

SC-19192: AN EVALUATION OF MUTAGENIC POTENTIAL  
EMPLOYING THE IN VIVO CYTOGENETICS METHOD IN THE RAT

P-T NO. 1027H72

FINAL REPORT

Submitted to

Searle Laboratories  
Chicago, Illinois



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**HAZLETON LABORATORIES**

*a subsidiary of Environmental Sciences Corporation*

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**SPONSOR:** Searle Laboratories

**DATE:** August 29, 1972

**MATERIAL:** SC-19192

**SUBJECT:** FINAL REPORT  
An Evaluation of Mutagenic Potential Employing the In Vivo  
Cytogenetics Method in the Rat  
Project No. 700-267

#### SUMMARY

SC-19192 was administered orally (intragastric) to four groups of 10 male albino rats each for five consecutive days, at dose levels of 0.25, 0.5, 1.0, and 2.0 g/kg/day given in three equally divided daily doses. One group of 10 rats served as a control and received the Tween 80-water vehicle; a sixth group served as a positive control and received a single intraperitoneal dose of 0.5 mg/kg of triethylenemelamine a known mutagen. Twenty-four hours after the last dose, each animal was administered Colcemid to arrest mitosis, was sacrificed, and bone marrow cells prepared and evaluated for chromosome mutations.

Evaluation of chromosome spreads indicated that SC-19192 did not alter (increase) the normal aberration frequencies observed in the control rats, and is thus not mutagenic. All data obtained were within normal limits. Triethylenemelamine was shown to be a potent mutagen in this test system, producing significantly increased aberration frequencies, and thus confirming the operational utility of the test system.



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## INTRODUCTION

The purpose of this study was to evaluate the mutagenic potential of SC-19192 employing the in vivo cytogenetic method in the rat.

## MATERIAL

Identification SC-19192; Lot No. 3R-A-7273.

Description A white, lumpy powder with no noticeable odor.

Received June 6, 1972.

Purity Assumed to be 100% pure.

## METHOD

### Animal Groups

Sixty healthy male albino rats of the Purina Caesarean-derived strain, approximately eight weeks of age at initiation of treatment, were selected at random and divided into the following groups:



<u>Group No.</u>	<u>Treatment</u>	<u>No. of Animals</u>	<u>Dosage Level*</u>
1	Vehicle Control	10	40 ml/kg/day
2	Positive Control (Triethylenemelamine)	10	0.5 mg/kg**
3	SC-19192	10	0.25 g/kg/day
4	SC-19192	10	0.5 g/kg/day
5	SC-19192	10	1.0 g/kg/day
6	SC-19192	10	2.0 g/kg/day

\* Administered intragastrically in three equally divided doses every three hours.

\*\* Administered intraperitoneally as a single dose on the final day of treatment for the other groups.

#### Compound Preparation

An aqueous vehicle of 1% Tween 80 in distilled water was employed. The SC-19192 was prepared fresh daily as a 10% suspension (w/v) in the aqueous vehicle. The positive control material (triethylenemelamine) was prepared as 0.5 mg/ml suspension in the same vehicle.

#### Procedure

Twenty-four hours after the fifth and last day of treatment, all rats received an intraperitoneal injection of Colcemid in distilled water (4.0 mg/kg), in order to arrest mitosis in dividing cells. Five hours after administration of the Colcemid, the rats were sacrificed by ether overdose.



#### Preparation of Chromosome Spreads

Immediately after sacrifice, bone marrow cells were collected from both femurs of each rat by aspiration into Hank's balanced salt solution. After washing, the cells were treated with hypotonic KCl (0.055 M) for 30 minutes. Subsequent to one hour's fixation in methanol acetic acid (3:1), air-dried preparations were made and stained with Giemsa -  $\text{NH}_4\text{OH}$ .

#### Cytologic Evaluation

Only those slides showing high mitotic indices and good chromosome morphology were evaluated for aberration. Five hundred bone marrow spreads were examined from each group of 10 rats (50 spreads per rat).

### RESULTS

#### General Observations - In-Life Phase

The incidence and frequency of soft feces was slightly increased among the Group No. 6 animals when compared with the other treated groups, which exhibited an incidence similar to that of the controls.

One high level (Group No. 5) rat was found dead on Day 3; cause of death was not established at necropsy.

Body weight and food consumption data are presented in Figure No. 1. Rats treated with SC-19192 exhibited weight losses (mean weight losses ranged from 7 to 21 grams) during the five day period, which exceeded the very slight mean weight loss (5 grams) shown by the negative control. Treated animals, likewise, showed lower food consumption than did the controls. Body weight gains and food consumption data for the positive control group revealed values



greater than that experienced by the negative controls, which can probably be related to the lower frequency of handling of these animals during the five day period.

Figure No. 1 - Mean body weight and food consumption data ( $\pm$ s.d.) recorded during the five-day treatment with SC-19192.

<u>Group (Treatment)</u>	<u>Mean Body Weight (g.)</u>		<u>Mean Food Consumption (g.)</u>
	Day 0	Day 5	Five Days
1 (Negative Control)	220 $\pm$ 11	215 $\pm$ 20	70 $\pm$ 11
2 (Positive (Control)	233 $\pm$ 14	238 $\pm$ 25	79 $\pm$ 19
3 (Low Level)	238 $\pm$ 7	217 $\pm$ 13	57 $\pm$ 15
4 (Mid Level)	231 $\pm$ 9	210 $\pm$ 16	59 $\pm$ 16
5 (High Level)	220 $\pm$ 12	213 $\pm$ 14	65 $\pm$ 12
6 (Very High Level)	225 $\pm$ 15	208 $\pm$ 17	61 $\pm$ 9

#### Cytologic Evaluation of Chromosome Spreads

The type and incidence of aberrations found among individual animals is presented in Table No. 1. Means of total aberrations by group are given in Figure No. 2.

There was no indication of a compound-related effect upon the chromosomes of the animals treated with SC-19192. The mean aberration frequencies observed for the vehicle control and SC-19192 groups are within normal limits for the rat, based on historical control data from these laboratories.





Figure No. 2 - Mean aberration frequencies in bone marrow cell chromosomes for rats treated with SC-19192.

<u>Group (Treatment)</u>	<u>Mean Percentage of Cells With Aberration</u>
1 (Negative Control)	1.4 ± 1.89
2 (Positive Control)	50.2 ± 24.15 <sup>S+</sup>
3 (Low Dose)	1.2 ± 1.68
4 (Mid Dose)	2.4 ± 2.95
5 (High Dose)	2.2 ± 1.20
6 (Very High Dose)	2.2 ± 3.04

S+ = Significantly higher than control.

The aberration most frequently found in the chromosomes of bone marrow cells of both SC-19192-treated and vehicle-treated rats was the chromatid gap. A gap (the loss of chromosomal material resulting in a space less than one chromatid in width) is not considered a major chromosomal change, and is thus not included in the calculations. Other aberrations observed included breaks, deletions, telemere blebs, fragments, exchanges, and stickiness, and were seen at essentially comparable rates between control and treated animals.

Triethylenemelamine, a known mutagen employed as a positive control, was found to be mutagenic, exerting a statistically significant increase in major chromosomal aberrations.

Submitted by

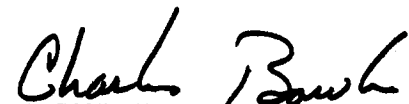


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Table No. 1 - Aberration frequencies observed in chromosome spreads of bone marrow cells from animals treated with SC-19192, triethylenemelamine (TEM), or which served as controls.

DOSE g/kg	RAT NO.	NO. OF CELLS	% CELLS W/GAPS*	% CELLS W/BREAKS	% CELLS W/ REUNION	% CELLS W/ >10 ABERRATION	% CELLS W/ ABERRATIONS*
Vehicle Control	1	50	0.00	0.00	0.00	0.00	0.00
	2	50	0.00	0.00	0.00	0.00	0.00
	3	50	0.00	0.00	0.00	0.00	0.00
	4	50	0.00	0.00	0.00	0.00	0.00
	5	50	0.00	0.00	0.00	0.00	0.00
	6	50	0.00	2.00	0.00	0.00	2.00
	7	50	0.00	0.00	0.00	0.00	0.00
	8	50	0.00	4.00	0.00	0.00	4.00
	9	50	0.00	4.00	0.00	0.00	4.00
	10	50	0.00	4.00	0.00	0.00	4.00
MEAN			0.00	1.40	0.00	0.00	1.40
TEM	11	50	0.00	2.00	6.00	54.00	62.00
	12	50	0.00	4.00	2.00	64.00	70.00
	13	50	0.00	10.00	6.00	66.00	82.00
	14	50	0.00	6.00	8.00	50.00	64.00
	15	50	0.00	2.00	8.00	28.00	38.00
	16	50	2.00	8.00	12.00	30.00	50.00
	17	50	0.00	2.00	0.00	0.00	0.00
	18	50	4.00	0.00	8.00	60.00	68.00
	19	50	8.00	18.00	8.00	6.00	32.00
	20	50	8.00	8.00	4.00	24.00	36.00
MEAN			2.20	6.00	6.20	36.40	50.20

\*The frequencies of chromosomes with gaps are not included in the calculation of total percentage of aberrations.

Table No. 1 - (Continued)

<u>DOSE</u> <u>g/kg</u>	<u>RAT NO.</u>	<u>NO. OF</u> <u>CELLS</u>	<u>% CELLS</u> <u>W/GAPS*</u>	<u>% CELLS</u> <u>W/BREAKS</u>	<u>% CELLS W/</u> <u>REUNION</u>	<u>% CELLS W/</u> <u>&gt;10 ABERRATION</u>	<u>% CELLS W/</u> <u>ABERRATIONS*</u>
0.5	21	50	0.00	0.00	0.00	0.00	0.00
	22	50	0.00	0.00	0.00	0.00	0.00
	23	50	0.00	0.00	0.00	0.00	0.00
	24	50	0.00	0.00	0.00	0.00	0.00
	25	50	0.00	0.00	0.00	0.00	0.00
	26	50	6.00	0.00	4.00	0.00	4.00
	27	50	0.00	2.00	0.00	0.00	2.00
	28	50	0.00	0.00	0.00	0.00	0.00
	29	50	2.00	2.00	0.00	0.00	2.00
	30	50	0.00	2.00	0.00	2.00	4.00
MEAN			0.80	0.60	0.40	0.20	1.20
1.0	31	50	0.00	2.00	0.00	0.00	2.00
	32	50	0.00	0.00	0.00	0.00	0.00
	33	50	0.00	0.00	0.00	0.00	0.00
	34	50	0.00	0.00	0.00	0.00	0.00
	35	50	2.00	4.00	0.00	0.00	4.00
	36	50	0.00	0.00	0.00	0.00	0.00
	37	50	0.00	8.00	0.00	0.00	8.00
	38	50	0.00	6.00	0.00	0.00	6.00
	39	50	0.00	0.00	0.00	0.00	0.00
	40	50	0.00	4.00	0.00	0.00	4.00
MEAN			0.20	2.40	0.00	0.00	2.40

\*The frequencies of chromosomes with gaps are not included in the calculation of total percentage of aberrations.

Table No. 1 - (Continued)

<u>DOSE</u> <u>g/kg</u>	<u>RAT NO.</u>	<u>NO. OF</u> <u>CELLS</u>	<u>% CELLS</u> <u>W/GAPS*</u>	<u>% CELLS</u> <u>W/BREAKS</u>	<u>% CELLS W/</u> <u>REUNION</u>	<u>% CELLS W/</u> <u>&gt;10 ABERRATION</u>	<u>% CELLS W/</u> <u>ABERRATIONS*</u>
2.0	41	50	0.00	4.00	0.00	0.00	4.00
	42	50	2.00	2.00	0.00	0.00	2.00
	43	50	2.00	2.00	0.00	0.00	2.00
	44	50	2.00	4.00	0.00	0.00	4.00
	45			ANIMAL DIED			
	46	50	2.00	0.00	0.00	0.00	0.00
	47	50	0.00	2.00	0.00	0.00	2.00
	48	50	0.00	2.00	0.00	0.00	2.00
	49	50	0.00	2.00	0.00	0.00	2.00
	50	50	0.00	2.00	0.00	0.00	2.00
MEAN			0.80	2.23	0.00	0.00	2.23
4.0	51	50	6.00	2.00	0.00	0.00	2.00
	52	50	0.00	2.00	0.00	0.00	2.00
	53	50	2.00	0.00	0.00	0.00	0.00
	54	50	4.00	4.00	0.00	0.00	4.00
	55	50	0.00	2.00	0.00	0.00	2.00
	56	50	0.00	10.00	0.00	0.00	10.00
	57	50	0.00	2.00	0.00	0.00	2.00
	58	50	0.00	0.00	0.00	0.00	0.00
	59	50	0.00	0.00	0.00	0.00	0.00
	60	50	0.00	0.00	0.00	0.00	0.00
MEAN			1.20	2.20	0.00	0.00	2.20

\*The frequencies of chromosomes with gaps are not included in the calculation of total percentage of aberrations.